

Prevalence of antibodies to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma hominis* in infertile women

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SUMMARY A total of 57 infertile women, who had been referred for in vitro fertilisation or for diagnostic laparoscopy, were tested for the presence of antibodies to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma hominis*. Four were excluded from the study. Of the remaining 53, 33 had laparoscopically obvious tubal disorders, such as adhesions, distal occlusions and strictures, and 20 did not. Antibodies to *C trachomatis* were found in 7/33 (21.2%) v 0/20, antibodies to *N gonorrhoeae* in 20/38 (60.6%) v 5/20 (25%), and antibodies to *M hominis* in 18/24 (75%) women with tubal disorders v 13/19 (68.4%) of those with no disorder. Antibodies to *C trachomatis* and *N gonorrhoeae* were significantly ($p < 0.05$) more common in women with tubal disorders. The high prevalence of antibodies to *N gonorrhoeae* in infertile women without tubal disorders suggests that ciliated tubal epithelium is damaged after inflammation without this being laparoscopically visible. Our results confirm the important role of *N gonorrhoeae* and *C trachomatis* in the aetiology of infertility after tubal inflammation.

Introduction

Salpingitis may lead to tubal infertility, the relative risk of which, after one or more episodes of salpingitis, varies between 12.8% and 75%.^{1,2} Moreover women who have had salpingitis have a seven-fold to tenfold risk of ectopic pregnancy. Tubal damage after infection is estimated to be an aetiological factor in 30% to 40% of infertile women.² Most cases of salpingitis are caused by the ascending spread of infection from the lower genital tract. Acute salpingitis has a polymicrobial aetiology. Anaerobic micro-organisms such as *Peptostreptococcus*, *Peptococcus*, *Clostridium*, and *Bacteroides* species have been isolated, either individually or in combination with *N gonorrhoeae*, *M hominis*, or *C trachomatis*.³ The relative importance of these organisms as causative agents has not been agreed. In the United States gonococci and anaerobes are more commonly associated with salpingitis than in

Sweden⁴ and France,⁵ where most isolates yield *C trachomatis*. These differences in the prevalence of causative organisms may be attributed to epidemiological factors, the length of time after infection, and the methods used for taking specimens for culture. Moore *et al* showed antibodies to *C trachomatis* in 73% of infertile women with distal tubal occlusion, but antibodies could not be detected in women with normal tubes.⁶ Punnonen *et al*⁷ and Conway *et al*⁸ made similar observations. These studies did not, however, evaluate antibodies to *M hominis* or *N gonorrhoeae*.

We undertook the study reported here to assess the relative importance of *N gonorrhoeae*, *M hominis* and *C trachomatis* as organisms causing tubal inflammation leading to infertility by measuring the prevalence of antibodies to these organisms in a group of infertile women.

Patients and methods

STUDY POPULATION

We tested serum from 57 women who had been infertile for at least 18 months and had been referred by their doctors to the gynaecological outpatient

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clinic of the University Hospital Rotterdam, the Netherlands for in vitro fertilisation or diagnostic laparoscopy.

CLASSIFICATION OF DISORDERS OF THE FALLOPIAN TUBES

We regarded the presence of peritubal adhesions, strictures, tubal occlusions, or a combination of these disorders as a macroscopic feature of tubal disease. This was the case in 33 women, two of whom showed only peritubal adhesions. The 20 women who did not show such abnormalities were considered to be infertile for unknown reasons. Four women had well documented causes of tubal disorders: one had had endometriosis, two had had tuberculous salpingitis, and one had had peritonitis after a perforated appendix. These four women were excluded from evaluation.

INVESTIGATIONS OF INFERTILITY

We recorded each patient's age and history of pregnancy, salpingitis, pelvic surgery, and the use of an intrauterine device (IUD). Infertility investigations consisted of semen analysis, basal temperature curve, postcoital test, hysterosalpingography, and laparoscopy with intrauterine injection of methylene blue to assess tubal patency.

SEROLOGY TESTS

We tested serum samples for the presence of antibodies to *C trachomatis* by the microimmunofluorescence technique of Wang *et al.*⁹ Six pooled antigens were used: A to C, DE, FG, I, J, and K. A titre of >1/8 was considered to be positive. Antibodies against gonococcal pili antigens were shown by an enzyme linked immunosorbent assay (ELISA), as described by Oranje *et al.*¹⁰ An extinction of >1.15 was considered to be positive. Anti-human immunoglobulin conjugate was used in both tests.

Antibodies to *M hominis* were shown by the indirect immunofluorescence method of Rosendal *et al.*¹¹ A strain of *M hominis* that had been isolated from a patient served as antigen. Positive control

serum was kindly supplied by Dr K Lind (Statens Serum Institut, Copenhagen, Denmark). Serum dilutions of 1/40 and 1/80 were prepared before the test. Anti-human IgG conjugate was used. Serum samples from 25 healthy women blood donors aged 22 to 34 were tested for the presence of the above mentioned antibodies.

STATISTICAL METHODS

The χ^2 method for independent samples was used to assess the level of significance.

Results

Table I shows that significantly more ($0.01 < p < 0.05$) women with macroscopically visible tubal disorders had antibodies to *C trachomatis* and to gonococcal pili in their serum than those without such disorders. Positive serology test results for chlamydiae were found in seven (13.2%) out of 53 women. In these seven women the most common serogroup was DE (in five women) followed by A-C (in four) and FG (in four), I (in three), and J (in two). In three out of these seven women only one antigen pool could be found. Antibodies to gonococcal pili antigen were present in 25 (47.2%) out of 53 women. Antibodies to *M hominis* were present in 31 (72%) out of 43 women, of whom 18 had tubal disorders and 13 did not. Of the 25 women blood donors, none had antibodies to *C trachomatis*, one had antibodies to gonococcal pili, and four had antibodies to *M hominis*.

Table II shows that antibodies to *C trachomatis* were significantly ($0.01 < p < 0.05$) more common in women with a history of salpingitis. Antibodies to gonococcal pili were also more common in such women, although not significantly so. Antibodies to *M hominis* were found more commonly in women with secondary infertility than in those with primary infertility. The number of women (nine) with a history of ectopic pregnancy was too small to allow statistical analysis. As the historical data on the use of IUDs were incomplete, these were not evaluated.

TABLE I Demographic, historical, and serological findings in relation to the condition of fallopian tubes as seen at laparoscopy

	Tubal disorder	No tubal disorder
Mean (SEM) age (years)	32.0 (0.7) (n = 33)	31.4 (0.9) (n = 20)
No (%) with secondary infertility	17/33 (51.5%)	9/20 (45%)
Mean (SEM) duration of infertility (years)	7.4 (0.8) (n = 31)	5.2 (0.7) (n = 18)
No (%) with history of salpingitis	14/20 (70%)	1/16 (6.3%)*
No (%) with chlamydial antibodies	7/33 (21.2%)	0/20 (0%)*
No (%) with gonococcal antibodies	20/33 (60.6%)	5/20 (25%)*
No (%) with chlamydial or gonococcal antibodies, or both	24/33 (72.7%)	5/20 (25%)*
No (%) with mycoplasmal antibodies	18/24 (75%)	13/19 (68.4%)

* $p < 0.001$; ** $0.01 < p < 0.05$.

TABLE II Prevalence of chlamydial, gonococcal, and mycoplasmal antibodies in infertile women related to historical data

	No (%) with chlamydial antibodies/No with or with no history		No (%) with gonococcal antibodies/No with or with no history		No (%) with chlamydial or gonococcal antibodies, or both/No with or with no history		No (%) with mycoplasmal antibodies/No with or with no history	
	With history	No history	With history	No history	With history	No history	With history	No history
History of:								
Salpingitis	5/14 (35.7)	1/23 (4.3)*	9/15 (60)	9/23 (39.1)	12/15 (80)	9/23 (39.1)*	9/14 (64.3)	13/21 (61.9)
Secondary infertility	4/29 (13.8)	2/27 (7.4)	12/29 (41.4)	14/27 (51.9)	14/29 (48.3)	15/27 (55.6)	21/27 (77.8)	12/24 (50)
Ectopic pregnancy	1/9 (11.1)	6/48 (12.5)	4/9 (44.4)	22/48 (45.8)	4/9 (44.4)	26/48 (54.2)	5/8 (62.5)	28/44 (63.6)

*0.01 < p < 0.05

Table III shows that the predictive value for the presence of macroscopically visible tubal disorders in serum containing chlamydial or gonococcal antibodies, or both, ranged from 64.2% to 94.4%.

Discussion

The prevalence of antibodies to *C trachomatis* in our patients was low (13.2%) compared with that (about 30%) observed by Moore *et al.*⁶ Epidemiological differences in the prevalence of chlamydial infections in both populations might offer an explanation for this discrepancy. Moreover, the mean duration of infertility in women in our study with tubal disorders was 7 years and 4 months, and in women without such disorders it was 5 years and 2 months. The length of time after infection that antibodies to chlamydiae are demonstrable in man remains to be assessed. Mårdh reported the disappearance of IgG and IgM antibodies after 1 year and 5 months despite initial high titres.¹² The long duration of infertility may have affected our results. Moore *et al* found a mean duration of raised titres of four years, which was considerably less time than we found in our study.

The high prevalence of antibodies to gonococcal pili both in the total study population (47.2%) and in the women with tubal disorders (60.6%) was unexpected. Mårdh detected gonococcal antibodies in less than 18% of 60 women with laparoscopically confirmed salpingitis.⁴ Eschenbach *et al*, however, found positive serology test results in 84% of 38 patients with salpingitis and gonococcal cervicitis and in 36% of 35 patients without current gonorrhoea.³

Our findings suggest a higher prevalence of gonococcal than chlamydial infections at the time of salpingitis. In 248 women who attended the venereal diseases outpatient clinic in Utrecht, the Netherlands in 1979-80, *N gonorrhoeae* was isolated in 34%, *C trachomatis* in 15%, and *M hominis* in 63%.¹³ Gonococcal antibodies were found in 25% of infertile women without laparoscopically obvious tubal disorders. This figure was considerably higher than that for gonococcal antibodies in our blood donors, which may indicate that gross examination by laparoscopy does not detect all tubal abnormalities in patients with tubal infertility. In such patients, dysfunction of ciliated tubal epithelium after inflammation may only be detectable by histopathological investigations.^{14 15}

Antibodies to *M hominis* were shown in 72% of our study population. The prevalence, however, was similar in women with and without tubal disorders (table I). The role of *M hominis* as a causative organism of salpingitis needs to be established. Animal studies suggest that it has a role in the aetiology of parametritis, rather than salpingitis.¹⁷

The difference in prevalence of chlamydial antibodies in women with and without a history of salpingitis was striking (table II). Antibodies to gonococcal pili were also more common in women with a history of salpingitis than in those with no such history, but the difference was not significant. In general, salpingitis associated with chlamydiae has a clinically milder course than that caused by gonococci or anaerobes, which seldom remains unnoticed. We cannot explain our results, which contradict this fact.

Our study clearly suggests a role for sexually transmitted organisms in the aetiology of infertility after tubal inflammation. Prospective studies of women with salpingitis, well documented by laparoscopic and bacteriological data, are necessary to gain further insight into the role of several micro-organisms in the development of tubal infertility. The high predictive value of the presence of antibodies to *chlamydiae* or gonococcal pili, or both, for the presence of tubal pathology is worth mentioning.

TABLE III Predictive value of positive serology test results for tubal disorders, as seen at laparoscopy

Serology test results	Predictive value within 95% confidence limits
Chlamydial antibodies	59-100
Gonococcal antibodies	59.3-93.2
Chlamydial or gonococcal antibodies, or both	64.2-94.1

Positive serological test results may also be valuable in explaining the aetiology of infertility in women when this is not known. Testing for these antibodies should therefore form a part of every routine infertility investigation.

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